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Mustela or *Vison*? Evidence for the taxonomic status of the American mink and a distinct biogeographic radiation of American weasels

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ABSTRACT

The American mink's relationship to the weasels in *Mustela* has been uncertain. Karyological, morphological, and phylogenetic comparisons to Eurasian *Mustela* support placing the mink outside the genus as *Neovison vison*. However, genetic comparisons that incorporate other endemic American *Mustela* suggest the interpretation of *N. vison*'s position to *Mustela* has been handicapped by biased geographic sampling. Here, we analyzed mitochondrial cytochrome-*b* from all weasels endemic to the Americas, including two poorly known South American species (*M. felipei*, *M. africana*), weasels native to North America (*M. vison*, *M. frenata*, *M. nigripes*), *Mustela* migrant to North America (*M. erminea*, *M. nivalis*), palearctic *Mustela*, and other American members of Mustelidae. Bayesian and likelihood inference methods were used to construct a phylogeny of *Mustela*, and relaxed Bayesian phylogenetic techniques estimated ages of divergence within the genus using priors calibrated by fossil ages. Our analyses show that the American mink and the smaller *Mustela* endemic to the Americas represent a distinct phylogenetic heritage apart from their Eurasian cousins, and biogeographic barriers like the Bering and Panamanian land bridges have influenced the evolutionary history of *Mustela* in the Americas.

1. Introduction

In the oral traditions of native North Americans, the American mink (*Neovison vison*, hereafter *Mustela vison*) has many names. The Kwakiutl cultures of the Pacific Northwest had legends of 'Made-like-the-Sun' or 'Born-to-be-the-Sun,' a powerful, yet boastful male child of father Sun and mother Sea Lion who used trickery to kill and eat his brother, Young Seal, and also his best friend, Land Otter (Boas, 1887a,b). In Klamath customs, Sqel, or Old Mink, had his heart stolen by the daughter of Le*w (or Lao), a monstrous octopoidal creature dwelling in Crater Lake, and had to outwit and slaughter the aquatic beast to steal it back (Winthrop, 1997; NPS, 2001). The mink is also called a "son of the seaworld and the skyworld" (McWilliams, 1996) in myths from the Makah tribe of the Olympic peninsula because he dwelt on land but caught fish from the water (Lockard and Barry, 2003), and in the deep South of colonial America, Uncle Remus spun great tales of Brer Mink's fishing ability and how his pride got him swindled out of fish by Brer Tarrypin (Harris, 1883).

Just as the mink's name changes to reflect diverse cultural legends, the taxonomy of the American mink has also varied greatly with the analysis of different morphological and biochemical characters. Systematists have often expressed uncertainty that it might not even belong with other weasels in the genus *Mustela*, or if so, where within this lineage it fits (Youngman, 1982; Masuda and Yoshida, 1994; Baryshnikov and Abramov, 1997; Lariviére, 1999; Marmi et al., 2004; Koepfli et al., 2008). For example, analyses of chromosome G-banding, restriction enzymes, immunoglobin chains, and other biochemical characters have all maintained that *M. vison* should be phylogenetically outside the Eurasian *Mustela* (Graphodatsky et al., 1976; Belyaev et al., 1980; Brinck et al., 1983; Lushnikova et al., 1989; Taranin et al., 1991). In contrast, it was considered a close relative to the European mink, *M. lutreola*, as they share particular adaptive skeletal traits, and the two minks were often placed in the same subgenus *Vison* (Gray, 1843, 1865; Corbet, 1966; Heptner et al., 1967; Kurtén, 1968; Herán, 1970; Hoffmann, 1976; Youngman, 1982). Molecular evidence has demonstrated that the two minks are more distantly connected (Davison et al., 1999; Kurose et al., 2000; Sato et al., 2003, 2004; Flynn et al., 2005; Fulton and Strobeck, 2006; Koepfli et al., 2008), and morphological differences removed *M. lutreola* from *Vison* (Petrov, 1958), while bacular structure segregated *M. vison* into a proposed new subgenus *Neovison* (Baryshnikov and Abramov, 1997). *Neovison* was then elevated to generic status (Abramov, 2000). Characterization of the os penis was subsequently used by the same authors to revert the American mink's generic standing back to *Mustela* (Baryshnikov et al., 2003), although quickly thereafter, it was officially recognized as *Neovison vison* (Wozencraft, 2005).

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Despite nominal confusion surrounding the American mink, molecular evidence from within the genus *Mustela* indicates a basal split occurring between the American mink and other members of the genus that are distributed across Eurasia. A limited study of six *Mustela* species in Japan suggested that including *M. vison* would make the genus paraphyletic (Masuda and Yoshida, 1994), while a later study showed that the American mink was significantly different from and sister to weasels distributed across Eurasia, though not paraphyletic to the genus (Kurose et al., 2000). Other genetic studies with similar results incorporated both mitochondrial and nuclear genes, though most studies were limited in overall number of sampled species and/or were geographically biased by comparing Eurasian species to the American mink [Davison et al., 1999: 337 bases of cytochrome-*b* (cyt-*b*); Kurose et al., 2000: complete cyt-*b*; Hosoda et al., 2000: partial and complete cyt-*b*; Sato et al., 2003: IRBP and cyt-*b*; Marmi et al., 2004: complete cyt-*b*; Sato et al., 2004: IRBP, RAG1].

Mustela phylogenies have been geographically biased toward Eurasian species, with the American mink included primarily because it has been introduced to Europe and Asia. Broader sampling for phylogenetic familial structure has more often included a second American representative from *Mustela*, the long-tailed weasel (*M. frenata*). When both the American mink and the long-tailed weasel have been incorporated in molecular analyses, they fall out as sister taxa, forming a distinct arrangement separate from holarctically distributed and Eurasian *Mustela* species (Dragoo and Honeycutt, 1997: 12S and 16S rRNA; Koepfli and Wayne, 1998: complete cyt-*b* gene; Koepfli and Wayne, 2003: 5 nuclear genes; Flynn et al., 2005: 3 nuclear and 3 mitochondrial genes; Fulton and Strobeck, 2006: five nuclear genes; Koepfli et al., 2008: 20 nuclear genes + cyt-*b*). The separate assemblage of the two endemic American *Mustela* relative to their Eurasian congeners might thus suggest the possibility of a monophyletic group endemic to the New World and distinct from colonizing Old World lineages. Furthermore, the positioning of both American weasels relative to Eurasian *Mustela* proposes that restricted geographic sampling that omitted other American endemics has limited our understanding of the true evolutionary history of *M. vison*.

Seven extant species in the genus *Mustela* inhabit the Americas; they include the ermine (*M. erminea*), least weasel (*M. nivalis*), black-footed ferret (*M. nigripes*), American mink (*M. vison*), long-tailed weasel (*M. frenata*), and two poorly known South American species, the Colombian weasel (*M. felipei*) and tropical weasel (*M. africana*) (Fig. 1). Ermine and least weasels occupy high to mid-latitude environments in the Western Hemisphere (Kurtén, 1968; Savage and Russell, 1983). The black-footed ferret is restricted to North America, while the American mink and long-tailed weasel occupy ranges throughout North America, and both range into South America, where the mink has been introduced. The Colombian and tropical weasels have very limited distributions in South America that overlap or border the distribution of long-tailed weasels along the Andean Cordillera. The Columbian weasel resembles the American mink in dorsal coloration, and both South American species have interdigital webbing like *M. vison*, though they differ from *M. vison* and *M. frenata* in vertebral architecture (Izor and de la Torre, 1978) and from all other weasels in bacular structure (Izor and Peterson, 1985).

Historically, weasels have their origins in Eurasia. Ermine and least weasels have been found in fossil deposits in Asia, dating to the late Miocene (Fortelius, 2007, NOW public release 030717), while the black-footed ferret presumably had an ancestor that entered from Eurasia across the Bering land bridge (Anderson, 1973, 1977; Youngman, 1994; Davison et al., 1999). Fossil evidence for both the American mink and long-tailed weasel suggests their origins lie in North America. Although documented fossils of either South American weasel are lacking, their morphology suggests a close affinity to the long-tailed weasel (Hall, 1951; Holmes, 1988; Abramov, 2000), and Holmes (1988) assumed that *M. frenata* and the two southern weasels were American endemics. Izor and Peterson (1985) hypothesized that Grammogale, the subgenus containing *M. africana* and *M. felipei* (Youngman, 1982), had a much longer evolutionary history in South America than suggested by the closing of the Panamanian isthmus. They postulated that two distinct *Mustela* stocks were present in Central America when the land bridge was established; one lineage, likely ancestral to *M. frenata*, occupied the highlands, while the second lineage, ancestral to Grammogale, favored lowlands and river valleys.

Here, we use DNA sequences of the cyt-*b* gene as a means of estimating the particular affinities of the American mink and all its American congeners relative to those distributed across Europe and Asia. Because our focus is on relationships among species in a genus and not on broader familial relationships, a mitochondrial gene is the most appropriate marker for the evolutionary scale of interest (DeYoung and Honeycutt, 2005). Moreover, because broader geographic sampling with the inclusion of additional species has effectively increased resolution for phylogenetic studies within a genus (Garcia-Paris et al., 2000; Glor et al., 2001; Bellinvia, 2004), we incorporated genetic representatives from the American mink, a majority of Eurasian *Mustela*, and all members of the genus currently distributed throughout the Americas, including new data from the two species not previously genetically characterized, the tropical weasel, *M. africana*, and the Colombian weasel, *M. felipei*. Our results suggest that not only do the American mink and weasels represent a genetically distinct New World radiation, but also that they might deserve designation as an entirely separate genus from their Old World counterparts.

2. Materials and methods

2.1. Study organisms

For genetic analyses, we included representatives from all species of *Mustela* presently known to inhabit the Americas. Both Eurasian and American representatives of *M. erminea*, *M. nivalis*, and *M. vison* were incorporated into analyses. To decipher interspecific relationships within extant members of the genus *Mustela* and evaluate their monophyly as a genus, including the two newly characterized species, within the context of the American Mustelidae, we included complete mitochondrial sequences of the cyt-*b* gene from several other mustelid species whose modern ranges lie in the western hemisphere (Table 1). Most sequences were acquired from GenBank ($N = 25$), and the entire length of the cyt-*b* was sequenced for all new specimens from fresh tissues or museum skins ($N = 10$; Table 1). Since all three sequences were identical for the black-footed ferret, *M. nigripes*, only one representative was included in the phylogenetic analysis. Two procyonid species were also included (Table 1) for outgroup comparisons, as phylogenetic work suggests their sister relationship to the Mustelidae. New genetic sequences can be accessed through GenBank, GQ153570–GQ153579.

2.2. Phylogenetic analyses

For this analysis, we completed DNA extraction, amplification and sequencing of the entire cyt-*b* gene (1,140 bp). Cytochrome-*b* sequences reflect an appropriate scale of divergence times found within mustelids and have been successfully employed in previous phylogenetic studies (Brown et al., 1979; Wilson et al., 1985; Koepfli and Wayne, 1998, 2003). See Appendix S1 in Supplementary Materials for extraction and amplification procedures. A total of 28 species and 32 individuals were included in the analysis (Table 1).

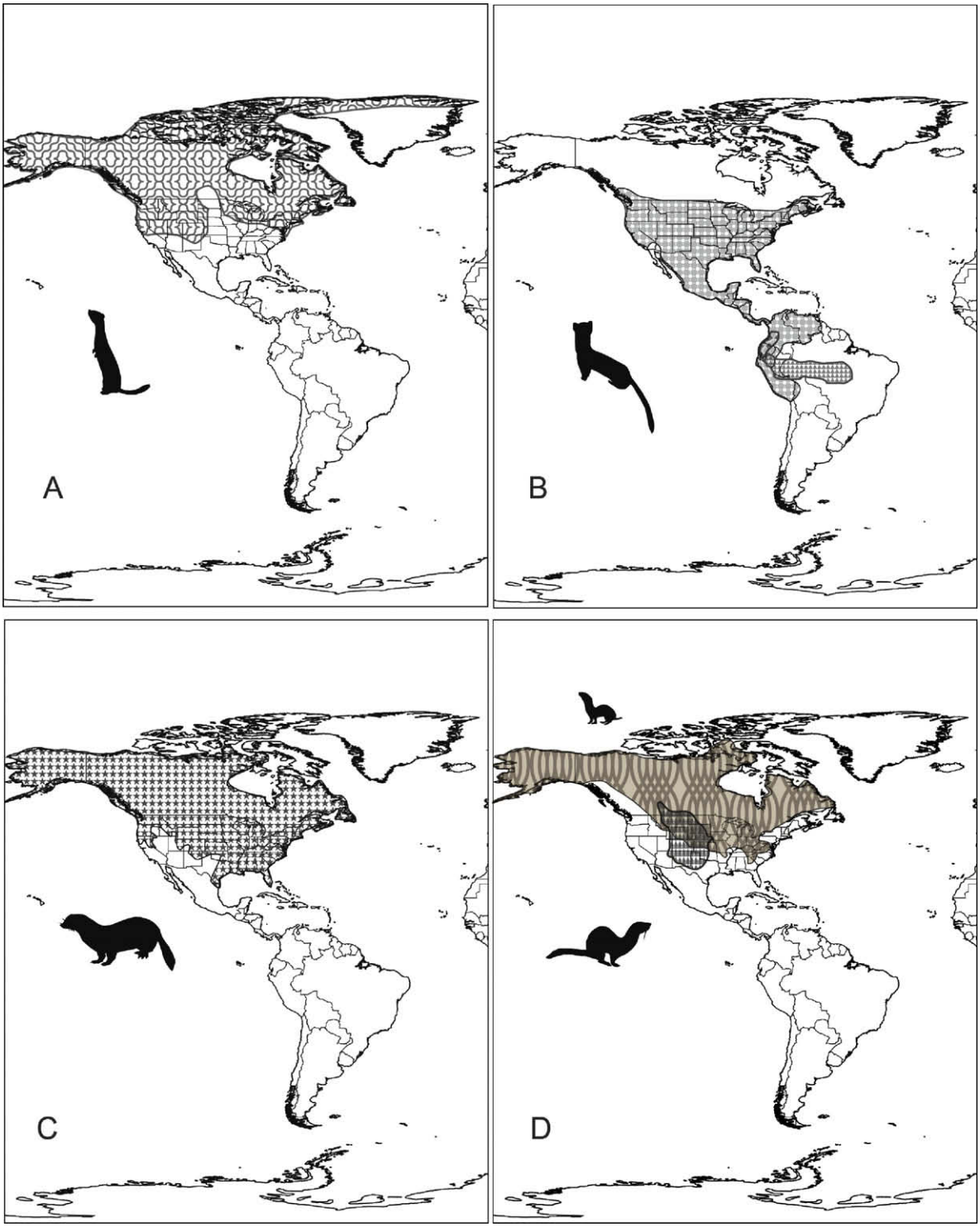

Fig. 1. Current distributions for all *Mustela* in the western hemisphere (Mollweide projection). Ranges are approximated from published species accounts for all *Mustela* except neotropical *M. africana* and *M. felipei*, whose ranges are estimated from museum records. (A) *M. erminea*, (B) *M. frenata* ●, *M. africana* ▲, *M. felipei* ◆, (C) *M. vison*, (D) *M. nigripes* ▲, *M. nivalis* ▒. Introductions of the American mink to South America are not depicted.

Absolute distances and pairwise differences in the cyt-*b* gene were calculated in PAUP* v.4.0 (Swofford, 2000), with pairwise comparisons using a corrected maximum likelihood distance with the general-time-reversible model (see below).

Phylogenetic trees were estimated from the data using criteria that established the best-fit models of molecular evolution. Model parameters were selected by the Akaike Information Criterion (AIC) in MrMODELTEST v2.2 (Nylander, 2004) for use in maximum likelihood (GARLI 0.96; Zwickl, 2006) and Bayesian inference (MRBAYES v3.01; Huelsenbeck and Ronquist, 2001). AIC selected for a general-time-reversible model (GTR) of base substitution, using empirical base frequencies, invariable sites ($I = 0.5194$), and a gamma distribution ($\Gamma = 1.7287$) with four rate categories. For maximum likelihood, we ran 3.0×10^6 generations with the

Table 1
Species (with abbreviations used in Table 2 and Supplementary Tables S2 and S3), common name, total number of sequences (number sequenced in this study), sequence or specimen ID, locality, and sources for sequences included in phylogenetic analysis of American Mustelidae, with emphasis on members of the genus *Mustela*. Abbreviations on specimen ID: X, AF, AB, DQ, EF are accession numbers from GenBank. FMNH is Field Museum of Natural History, MNHN is Muséum National d'Histoire Naturelle, Paris, France, MSB is Museum of Southwestern Biology, MVZ is Museum of Vertebrate Zoology, NK is New Mexico Kryovoucher (MSB), ROM is Royal Ontario Museum, UAM is University of Alaska Museum, USNM is US National Museum (Smithsonian), WiDNR is Wisconsin Division of Natural Resources.

Species	Common name	Total (N)	Sequence or specimen ID	Locality	Source
Procyonidae					
Procyon lotor (PLO)	Raccoon	1	X 94930	–	Ledje and Arnason (1996)
Bassariscus astutus (BAS)	Ringtail	1	AF 498159	–	Koepfli and Wayne (2003)
Mustelidae (*Mustelinae*)					
Gulo gulo (GGU)	Wolverine	1	UAM 53409	Alaska	Tomasik and Cook (2005)
Martes pennanti (MPE)	Fisher	1	AF 57131	Mass./Minn.	Koepfli and Wayne (1998)
Martes americana (MAM)	American marten	1	AF 57130	Wyoming	Koepfli and Wayne (1998)
Eira barbara (EBA)	Tayra	1	MSB 58756	Bolivia	Koepfli and Wayne (2003)
Galictis vittata (GVI)	Greater grison	1	MVZ 155226	Peru	Koepfli and Wayne (2003)
Galictis cuja (GCU)	Lesser grison	1	EF 987754	Argentina	Koepfli et al. (2008)
Mustela erminea (MER)	Ermine	2 (1)	AF 457445	Russia	Fleming and Cook (2002)
			UAM 99623	Washington	This study
Mustela altaica (MAL)	Mtn weasel	1	AB 26100	Mongolia	Kurose et al. (2000)
Mustela nivalis (MNI)	Least weasel	2 (1)	AF 457461	Unknown	Fleming and Cook (2002)
			WiDNR	Wisconsin	L. Ayers, Wisconsin DNR
Mustela itatsi (MIT)	Japanese weasel	1	AB 26104	Japan	Kurose et al. (2000)
Mustela sibirica (MSI)	Siberian weasel	1	AB 26108	Japan	Kurose et al. (2000)
Mustela nigripes (MNG)	Black-footed ferret	3 (3)	NK 39379	South Dakota	This study; tissues from
			NK 39380, NK 39382	Wyoming	USGS Biological Survey
Mustela lutreola (MLU)	European mink	1	AB 26105	Russia	Kurose et al. (2000)
Mustela eversmanni (MEV)	Steppe polecat	1	AB 26102	Russia	Kurose et al. (2000)
Mustela putorius (MPU)	European polecat	1	AB 26107	Russia	Kurose et al. (2000)
Mustela furo (MFU)	Domestic ferret	1	AB 26103	Domestic	Kurose et al. (2000)
Mustela vison (MVI)	American mink	2 (1)	AB 26109	Eurasia	Kurose et al. (2000)
			UAM 73639	Arkansas	This study
Mustela africana (MAF)	Tropical weasel	1 (1)	FMNH 106488	Brazil	This study
Mustela felipei (MFE)	Colombian weasel	1 (1)	FMNH 86745	Colombia	This study
Mustela frenata (MFR)	Long-tailed weasel	2 (2)	NK 122149,	Washington	This study
			TTU 44951	Mexico	This study
Mustela strigidorsa (MST)	Back-striped weasel	1	EF 987748	TC 284 At MNHN, France	Koepfli et al. (2008)
Mustela nudipes (MNU)	Malayan weasel	1	EF 987745	TC 403 At MNHN, France	Koepfli et al. (2008)
Mustelidae (Lutrinae)					
Lontra canadensis (LCA)	North American river otter	1	AF 57121	No locality	Koepfli and Wayne (1998)
Lontra longicauda (LLO)	Neotropical otter	1	AF 57123	Peru/Bolivia	Koepfli and Wayne (1998)
Lontra felina (LFE)	Marine otter	1	AF 57122	Chile	Koepfli and Wayne (1998)
Mustelidae (Taxidinae)					
Taxidea taxus (TTA)	American badger	1	AF 57132	California	Koepfli and Wayne (1998)

GTR + I + Γ model of evolution in GARLI 0.96 (Zwickl, 2006) multiple times to ensure that results were consistent, as the algorithm is stochastic (Zwickl, 2006). Branch support was estimated by performing 100 bootstrap replicates three times in GARLI to compare results for consistency. We also applied Bayesian techniques using the GTR + I + Γ model in Metropolis-coupled Markov chain Monte Carlo (MCMC) methods (MrBayes v. 3.2; Huelsenbeck and Ronquist, 2001). Two independent and simultaneous runs with four consecutive Markov chains proceeded for 3.0×10^6 generations each, with random starting trees for each chain, and tree sampling every 100 generations, resulting in a sample of 30,000 trees. Three chains were incrementally heated using a temperature scaling factor of $T = 0.2$ while the fourth ran cold. The first 25% of trees were discarded as a preliminary set of unstable generations, or burn-in, and the remaining trees were used to construct a 50% majority-rule consensus tree. We performed this analysis three times to ensure consistency of results, and nodes with posterior probabilities $\geqslant$95% were considered to be well supported. To investigate results of our phylogenetic analyses relative to published topologies, we constrained taxa to match that observed by Koepfli et al. (2008) and compared tree likelihood scores with the Shimodaira–Hasegawa test in PAUP* (Shimodaira and Hasegawa, 1999) using a maximum likelihood framework.

2.3. Divergence time estimates

The likelihood ratio test (LRT; Felsenstein, 1981) in PAUP* was applied to assess the appropriateness of applying a molecular clock to the cyt-*b* data. The likelihood scores of trees obtained both with and without enforcing a strict molecular clock were calculated under the best-fit substitution model (GTR + I + Γ). Results of the LRT suggested that the rates of substitution differed significantly among branches ($-\ln L_{\text{clock}} = 8828.52$ and $-\ln L_{\text{no clock}} = 8798.91$, 31 *df*, $p < 0.05$), indicating that the strict molecular clock model was inappropriate for our data.

In the absence of rate homogeneity, relaxed molecular clock models are appropriate alternative methods for estimating divergence dates, as these allow the rate of evolution to vary across the phylogenetic tree (Drummond et al., 2006). BEAST v1.4.6 (Drummond and Rambaut, 2006; Drummond et al., 2006) was used to apply a Bayesian relaxed molecular clock approach for estimating divergence times among lineages and clades (see Appendix S2 in Supplementary Materials for model parameters used).

To refine divergence estimates, fossil data were used to set probabilistic age calibration priors on specific nodes in the phylogenetic tree. Probabilistic priors are more appealing than fixed point calibrations (Drummond et al., 2006) because age estimates

on fossil materials inherently contain a level of uncertainty best represented by a flexible range of values (Ho, 2007; Leaché and Mulcahy, 2007). Since the appearance of a fossil in the geologic record necessarily postdates the origin of the group to which it belongs, fossils can only provide minimum age estimates for divergence events (Ho, 2007). A lognormal parametric distribution more appropriately models the uncertainty surrounding this minimum age than does a normal distribution because a normal distribution may also focus prior densities on ages that postdate the first appearance of a fossil in the geological record (Hedges and Kumar, 2004; Drummond et al., 2006; Ho, 2007; Leaché and Mulcahy, 2007). Nevertheless, for purposes of comparison to previous age estimates, we used priors sampled from both normal and lognormal distributions to estimate divergence ages. Parameters from the lognormal distribution were defined to incorporate 95% of the probability (Ho, 2007), with the highest probability being centered on the fossil age and the probability of divergence decreasing at older times (Leaché and Mulcahy, 2007).

Fossil calibrations used in the relaxed molecular clock approach incorporated the appearance of *Pseudobassaris* (28.0–28.5 Ma) and *Plesictis* (24.3–24.7 Ma) fossils to independently define the root and crown heights of the Mustelidae relative to Procyonidae. *Plesictis* represents the earliest known mustelid fossil (Wolsan, 1993, 1999; Sato et al., 2003), while *Pseudobassaris* contains synapomorphic cranial features common to both procyonids and mustelids and thus is believed to lie closest to the split of the two families (Wolsan, 1993, 1999; Wolsan and Lange-Badré, 1996; Sato et al., 2003). Calibration priors were set in two ways on the root and crown priors: (1) Using a normal distribution, we chose the older fossil appearance of *Pseudobassaris* (28.5 Ma) as the mean, with a standard deviation of 1.0, to represent the root height of the tree. Mean crown height for the Mustelidae was represented by the younger appearance of *Plesictis* (24.3 Ma) in the fossil record, also with a standard deviation of 1.0. (2) Prior values were chosen from a lognormal distribution to estimate root height using the appearance of *Pseudobassaris* such that the highest probability in the distribution occurred in the Oligocene (27.4–29.6 Ma; mean = 3.35, stdev = 0.5, zero offset = 5.0, initial value = 20.0). Prior distribution for the crown height of Mustelidae was set so that 95% of the values surrounded the appearance of *Plesictis* in the late Oligocene (23.4–25.3 Ma; mean = 3.19, stdev = 0.02, zero offset = 0.01).

The appearance of three other fossil groups was also used to calibrate divergence dates under normal and lognormal prior distributions. (A) Early *Mustela* fossils from Eurasia have been found in deposits dating from the late Miocene into the Pliocene (Fortelius, 2007, NOW public release 030717). Values for a prior on *Mustela* from a lognormal distribution were chosen so that they roughly represented this range of time, with the highest probability at about 5.3 Ma (mean = 1.668, stdev = 0.25, zero offset = 0.5). For the normal distribution, the mean was chosen at 5.3 Ma, with a standard deviation of 1.0 (Koepfli et al., 2008). (B) The oldest *M. erminea* fossils are estimated about 1.8 Ma (King, 1983). Values for the ermine group from a lognormal distribution were chosen using 1.8 Ma as the median (mean = 0.588, stdev = 0.3, zero offset = 0.08) such that the time span represented was the late Pliocene to the early Pleistocene. Values in the normal distribution had a mean of 1.8 Ma, with a standard deviation of 1.0. (C) The earliest *M. frenata* fossils are dated at about 1.9 Ma (Cassiliano, 1999; Alroy, 2002). We assigned a lognormal prior centered at 1.9 Ma and sampled a time spanning the late Pliocene to the Pleistocene (mean = 0.65, stdev = 0.3, zero offset = 0.02). For the normal distribution, we used a mean of 1.9 Ma, with a standard deviation of 1.0.

The Great American Interchange likely had a significant influence on the evolutionary history of *Mustela* in the Americas (Marshall, 1988; Webb, 1991). Assuming that mustelids were absent in South America prior to the existence of a land bridge (Marshall, 1988), it is still unclear whether diversification between *Mustela* species preceded colonization so probabilistic age priors with a normal distribution would appropriately model this uncertainty (Ho, 2007). Therefore, to estimate nodal age for the split between North and South American *Mustela*, we set calibration priors to represent the mean age of the formation of the Panamanian isthmus at 3.0 Ma, with a standard deviation of 0.5 Ma (Weinstock et al., 2005; Ho, 2007) in all analyses.

3. Results

3.1. Nucleotide variation

Absolute nucleotide differences and maximum likelihood genetic distances were greatest between families, expectedly more similar between genera in the same family (Supplementary Tables S1 and S2), and smallest between species in the same genus (Table 2 and S1). Genera differed by as little as 17.1% and as much as 30.2%, and, excluding *Mustela*, dissimilarity ranged from 6.1–17.4% among members of the same genus.

For the genus *Mustela*, overall nucleotide dissimilarities were similar to those found within other genera of the Mustelidae. Molecular variation ranged from 3 to 172 bases and ML distances

Table 2
Maximum likelihood differences with GTR model (below diagonal) and absolute basepair differences (above diagonal) for *Mustela* species. *Where more than one specimen has been included in the phylogenetic analysis [(2, MVI), (2, MFR), (2, MER), (2, MNI)], only the values for animals representing the ancestral clade have been reported here. For MFR, reported values are for TTU 44951, representing an animal from the middle of the current range. See Table 1 for abbreviations. Light gray box enclosing weasel species with an American distribution; medium gray includes weasel species with holarctic/Eurasian distributions. Dark gray box contains larger 'ferret' species in temperate regions of Eurasia.

	MVI*	MAF	MFE	MFR*	MER*	MAL	MNI*	MIT	MSI	MNG	MLU	MEV	MPU	MFU	MST	MNU
MVI*	–	125	114	112	129	147	152	134	141	145	141	140	139	141	141	154
MAF	0.136	–	82	75	132	140	151	146	141	146	144	139	137	139	153	172
MFE	0.123	0.082	–	73	120	135	144	127	141	140	135	134	132	135	140	155
MFR*	0.121	0.073	0.073	–	124	143	142	130	142	135	135	134	132	135	146	164
MER*	0.143	0.145	0.129	0.135	–	82	89	88	94	96	92	95	95	96	134	142
MAL	0.164	0.151	0.146	0.157	0.082	–	84	114	94	104	95	89	89	90	156	163
MNI*	0.172	0.166	0.158	0.154	0.090	0.083	–	95	95	97	93	94	94	95	132	163
MIT	0.148	0.163	0.139	0.141	0.090	0.122	0.096	–	60	63	59	64	63	65	143	154
MSI	0.157	0.154	0.156	0.156	0.097	0.095	0.096	0.058	–	51	40	41	45	42	151	158
MNG	0.162	0.161	0.155	0.146	0.098	0.107	0.098	0.061	0.048	–	31	32	36	33	151	163
MLU	0.157	0.158	0.148	0.147	0.094	0.096	0.093	0.057	0.037	0.028	–	9	13	12	145	160
MEV	0.155	0.151	0.147	0.145	0.097	0.089	0.094	0.063	0.038	0.029	0.008	–	4	3	144	159
MPU	0.154	0.148	0.144	0.143	0.098	0.089	0.094	0.061	0.042	0.033	0.012	0.004	–	7	143	159
MFU	0.157	0.151	0.149	0.147	0.099	0.090	0.096	0.064	0.039	0.030	0.011	0.003	0.006	–	144	160
MST	0.159	0.175	0.157	0.166	0.149	0.176	0.142	0.159	0.170	0.168	0.160	0.159	0.158	0.159	–	126
MNU	0.175	0.202	0.177	0.191	0.158	0.186	0.188	0.174	0.179	0.185	0.182	0.181	0.181	0.182	0.136	–

differed one percent or less among the *Putorius* (*M. lutreola*, *M. eversmanni*, *M. furo*, *M. putorius*) ferret group to 20.2% between the Malayan weasel, *M. nudipes*, and *M. africana*. Species with holarctic or Eurasian distributions were more similar to each other than to *Mustela* with strictly American distributions (Table 2). The one exception to this pattern was the black-footed ferret, *M. nigripes*, which now occupies a contracted American range but is more closely related to the ferrets and minks in Europe and Asia. *Mustela* primarily confined to Southeast Asia (the back-striped weasel, *M. strigidorsa*, and *M. nudipes*) were most divergent from all other *Mustela*, with ML differences ranging from 13.6% between the two species to 20.2% with one of the American weasels. Maximum likelihood differences between American weasels, including *M. vison*, and Eurasian weasels and 'ferrets' were 12.9–17.2% and 13.9–16.3%, respectively. The smaller-bodied Eurasian weasels deviated from the larger 'ferrets' 8.9–12.2%, yet dissimilarity within each group was generally less than 9.0%. Within the American weasels, the American mink was most highly differentiated (12.1–13.6% ML difference), while the American weasels did not vary more than 8.2%.

3.2. Phylogenetic structure

Phylogenetic analyses of maximum likelihood and Bayesian inference yielded identical tree topologies for the arrangement of *Mustela* (Fig. 2), though positions differed for *Eira barbara* relative to the *Martes* species and had low branch support. Genetic structuring in *Mustela* and within the family was generally consistent with

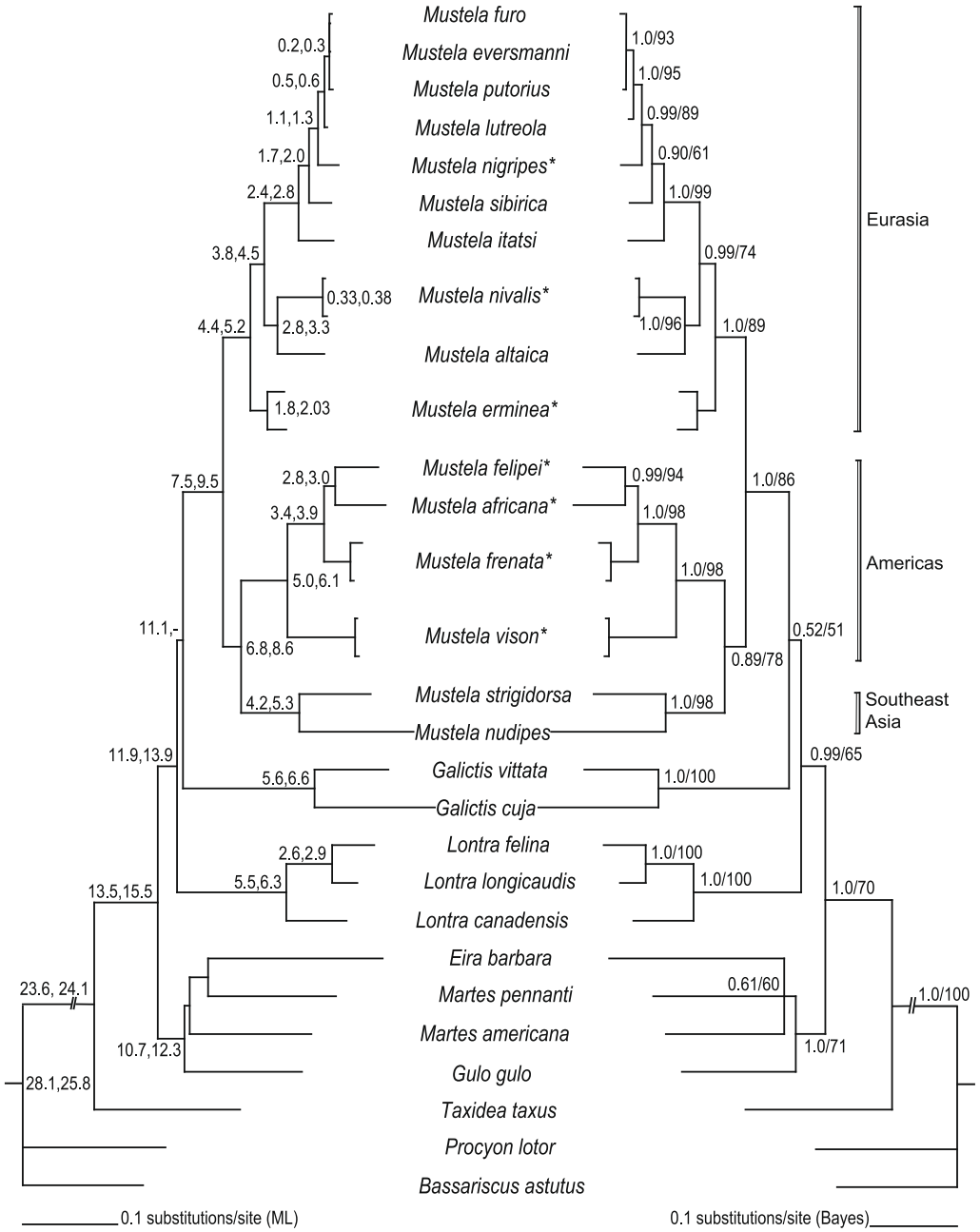

Fig. 2. Phylogenetic trees produced by maximum likelihood and Bayesian analysis of American mustelids. '*' represents *Mustela* species with current distributions in the Americas. Shown on the left-hand side of tree are the estimated mean ages of divergence (Ma) from both normal and lognormal distributions, respectively; tree topology from ML Garli analyses. On the right-hand side of tree are the Bayesian posterior probabilities/ML bootstrap values; tree topology reflects Bayesian analyses. Scale represents substitutions/site on both topologies.

former studies (see Koepfli et al., 2008, and references therein), except that *M. strigidorsa* and *M. nudipes* formed a clade with low branch support that was sister to the American weasels, though comparisons made between the likelihood scores of our optimal tree and a constraint tree following the topology in Koepfli et al. (2008) with the Shimodaira–Hasegawa (1999) test in PAUP* proved non-significant ($-\ln L_{optimal} = 9262.73$, $-\ln L_{constraint} = 9264.50$; $p = 0.293$).

Tree structure revealed two primary clades in *Mustela* (Fig. 2) that reflected not only genetic distinction, but also morphology and geography. One clade with higher branch support [1.0 posterior probability (PP), 89% bootstrap support (BS)] consisted of species with Eurasian origins, with the smaller weasels distinguished at the base of a larger 'ferret' group (Sato et al., 2003; Koepfli et al., 2008). A second clade with lower support (0.89 PP, 78 BS) contained two subclades, each with higher nodal support. One included an American cluster (1.0 PP, 98 BS) with the larger-bodied American mink markedly sister to a clade dividing the smaller long-tailed weasel from the South American tropical and Colombian weasels. A second sub-clade held *M. strigidorsa* and *M. nudipes* from Southeast Asia (1.0 PP, 98 BS). These geographic partitions were contrasted only by the lack of separation between the black-footed ferret of North America and the polecats and ferrets of Eurasia (Fig. 2).

3.3. Divergence estimates

Segregation between species was also reflected in the rates of molecular evolution. Estimates of divergence times using the uncorrelated relaxed lognormal molecular clock model (Drummond and Rambaut, 2006) evaluated rate heterogeneity among lineages. In the three independent runs with normal and lognormal priors, respectively, both the averaged standard deviation of the model ($\sigma_{norm} = 0.455$, 95% highest posterior density [HPD] = 0.313–0.608; $\sigma_{ln} = 0.420$, 95% HPD = 0.259–0.583) and the coefficient of variation ($\sigma_{r\ norm} = 0.433$, 95% HPD = 0.296–0.582; $\sigma_{r\ ln} = 0.400$, 95% HPD = 0.246–0.558) suggested that there was rate heterogeneity among lineages in the data, thus confirming the LRT results in PAUP*. All effective sample sizes in the normal and lognormal analyses were large (>1000), and measures of covariance suggested no autocorrelation of rates in the phylogeny (combined $mean_{norm} = 0.064$, 95% HPD = −0.183 to 0.304; combined $mean_{ln} = 0.033$, 95% HPD = −0.209 to 0.279).

Node priors placed on the phylogeny did not appear to unduly influence estimates of divergence times in either normally or lognormally distributed models (Supplementary Table S3). For most of the divisions in Mustelidae, the lognormal distribution yielded older mean divergence estimates than the normal distribution, although estimations from both types of priors were generally contained in the Miocene–Pliocene (Fig. 2). Our estimates overlapped with probable dates of divergence in previous studies using cyt-*b* (Kurose et al., 2000; Sato et al., 2003), but they are older than estimates in Koepfli et al. (2008), which used 20 nuclear genes in combination with cyt-*b*.

Normal and lognormal estimates on the root height of the tree spanned 25.8–28.1 Ma (Fig. 2 and Table S4). Divergence times for the basal split of the American badger, *Taxidea taxus*, from the rest of the represented Mustelidae were estimated at 23.6–24.1 Ma, while most generic divisions took place in the middle to late Miocene (~7.5–15.5 Ma). The primary separation of *Mustela* species in Eurasia from those in Southeast Asia and the Americas (7.5–9.5 Ma) overlapped the divergence of American species from Southeast Asian species in the late Miocene (6.8–8.6 Ma). Widespread differentiation between *Mustela* species occurred in the Pliocene, with the distinction of all the American species, as well as the partitioning of large and small *Mustela* species in Eurasia.

4. Discussion

4.1. Mustela in the Americas

The American mink, together with the American weasels, belongs to a distinct New World lineage. Cladogenesis portrays two divergent biogeographical lineages, with one across Eurasia and one in the Americas. Prior family-level mitochondrial phylogenies suggest that the American mink is sister to the long-tailed weasel in the New World (Dragoo and Honeycutt, 1997; Koepfli and Wayne, 1998, 2003; Flynn et al., 2005), and recent extensive nuclear evidence corroborates the presence of a distinct American group (Fulton and Strobeck, 2006; Koepfli et al., 2008).

Given this new genetic topology, there appear to have been at least four primary migrations of *Mustela* into the Americas. Our genetic evidence proposes that the earliest event resulted in the predecessor(s) to American *Mustela* crossing from Siberia into the New World during the late Miocene (8–10 Ma), while the other three events represent congeneric Pleistocene immigrations to the Americas from the Old World. Subsequent radiation occurred with the first wave, producing the endemic American mink and long-tailed, Colombian, and tropical weasels (see also Hosoda et al., 2000; Marmi et al., 2004; Koepfli et al., 2008). *Mustela* fossils first appear in North America in the late Miocene to early Pliocene (4.6–5.9 Ma, Tedford et al., 2004), a time that overlaps our estimates of the split between the larger mink and its smaller weasel relatives, and both *M. vison* and *M. frenata* appear distinctly in North American Pleistocene fossil deposits (Hibbard, 1970; Kurtén and Anderson, 1980; Sheffield and Thomas, 1997; Cassiliano, 1999; Lariviére, 1999; Alroy, 2002), though their fossil dates are much younger than our estimated genetic ages. However, little is known about the history of *Mustela* in the southern hemisphere, as fossil evidence for *M. frenata* in South America is scant and no fossils have been recovered for either species native to the southern neotropics. Collectively, *Mustela* were assumed to have crossed between North and South America during the Great Biotic Interchange (Marshall, 1988; Webb, 1991), though our estimates for divergence of *M. africana* and *M. felipei* from the long-tailed weasel (3.4–3.9 Ma) predate the closing of the Isthmus of Panama (2.5–3.0 Ma, Patterson and Pascual, 1968; Marshall, 1988; Webb, 1991), and favor a longer history to *Mustela* in tropical America (Hall, 1951; Izor and Peterson, 1985). Observed morphological departures (e.g., naked foot soles with extensive interdigital webbing; Izor and de la Torre, 1978) in the neotropical weasels from the long-tailed weasel favor the idea that *M. africana* arose from 'waif' *M. frenata* forms that crossed the Panamanian isthmus prior to its closure and evolved in isolation (Hall, 1951). These variant morphologies also suggest that two distinct weasel stocks were present in tropical Central America when the Panamanian land bridge was established (Izor and de la Torre, 1978), with one adapted for terrestrial life and the other more tailored to tropical cloud forest and aquatic environments (Schreiber et al., 1989; Alberico, 1994). Thus, the neotropical weasel species might realistically have been waif forms that dispersed from island to island along a growing archipelago from Central America (Coates and Obando, 1996).

Later waves of migration represent more recent Pleistocene invasions by *Mustela* species that originated in Eurasia. Among the small weasels, our estimates and others (Fleming and Cook, 2002; Martínková et al., 2007) indicate that ermine diverged close to 2 Ma, near the appearance of the first *M. erminea* fossils 1.8 Ma (King, 1983); successive fossil evidence suggests that one lineage migrated from its origin in Eurasia across Beringia into the Western Hemisphere approximately 1.2 Ma (Anderson, 1989), where ermine appear in the geological record of North America during

the Illinoian (Kurtén, 1966). Our results suggest that Eurasian and North American lineages of least weasels diverged more recently (330,000–380,000 ybp) and fossils indicate that one line emigrated across the Bering land bridge during the Wisconsinan glacial period (Kurtén and Anderson, 1980). Fossil remains specify that the larger black-footed ferrets evolved from Eurasian polecat stocks during the early Pleistocene (~1.5 Ma) (Anderson, 1973, 1977; Hillman and Clark, 1980; Anderson et al., 1986; Youngman, 1994), a time rather close to our divergence dates (1.1–1.3 Ma), and their movements into the Great Plains were likely facilitated by the presence of ice-free corridors during interglacial epochs of the Pleistocene (Hillman and Clark, 1980; Youngman, 1994).

The divergence estimates we report here are most often greater or equal to known fossil evidence, but usually contemporaneous with overlapping nodes in earlier work. General differences in divergence between molecular and fossil dates might be explained by the trend for gene evolution to predate species evolution (Graur and Li, 2000) or the taphonomic uncertainty that brackets a fossil age estimate. As for reported molecular dates, divergence ages for several nodes in our tree are within the same time frame as prior phylogenetic work (Koepfli and Wayne, 1998; Hosoda et al., 2000; Kurose et al., 2000; Sato et al., 2003; Marmi et al., 2004), though they assumed a constant rate of molecular evolution over the whole phylogeny and used one to a few fossils to calibrate mitochondrial rates of evolution. These comparisons come with the caveat that agreement or overlap of mean divergence times using constant or relaxed molecular clocks should not necessarily be viewed as corroborative evidence, as the assumptions made by these methods differ in many fundamental ways (Drummond et al., 2006).

Even when methodologies are similar, divergence estimates may be inconsistent due to differences in character and/or taxon sampling. We know of only one other study that has used comparable relaxed clock methods and fossil evidence to calibrate divergence estimates (Koepfli et al., 2008), and with few exceptions, time estimates were younger than times reported in this study. For instance, node age on the split between the American *Mustela*, which included *M. vison* and *M. frenata*, and Eurasian species occurred 5.2–6.6 Ma (Koepfli et al., 2008), while it was earlier for our estimates (7.5–9.5 Ma). Additional estimates with similar root and crown height priors aged the split between the two American taxa at 2.6–2.7 Ma in their study, but at 5.0–6.1 Ma in our study. These differences in age are not unexpected, given the data and taxa used. Koepfli et al. (2008) incorporated 20 nuclear genes together with cyt-*b* and eight fossil calibration points and several additional mustelid species, while our study used only cyt-*b*, five fossil calibrations, focused primarily on the species in *Mustela*, and included only American mustelid species in the phylogeny. Perhaps the most critical difference is accounted for when one considers that mitochondrial protein-coding genes have higher rates of mutation, and thus higher rates of substitution, so our older estimates are to be expected based on the different rates of molecular evolution observed between mitochondrial and nuclear loci.

4.2. Sisters?

The phylogenetic relationship of the weasels in Southeast Asia to those in the Americas or mainland Europe and Asia should be interpreted with caution. Our molecular results suggest that *M. strigidorsa* and *M. nudipes* belong to an independent lineage in Southeast Asia that differs less from the American weasels than from other Eurasian weasels. In support of their affinity to the American weasels, Youngman (1982) observed that bacular anatomy in the Malayan weasel was similar to that of the Columbian weasel, though he did not include both species in his morphological dendrograms. Later, in a more inclusive morphological assessment, Holmes (1988) grouped *M. strigidorsa* and *M. nudipes* with the European mink, the Siberian weasel (*M. sibirica*), and the Indonesian mountain weasel (*M. lutreolina*), apart from the American species. However, while our topology using cyt-*b* produced a lower log likelihood score, it was not significantly different from a topology of cyt-*b* and nuclear data that constrained a relationship between Eurasian and Southeast Asian *Mustela* species (Koepfli et al., 2008), and node support for this relationship was much higher than in our study, though *M. africana* and *M. felipei* were absent. This suggests that there is genetic distinction between the majority of Eurasian *Mustela* species and those in Southeast Asia, and the addition of other weasel species from Asia and/or genetic information on the South American species would help to elucidate the appropriate phylogenetic relationships between *Mustela* in Southeast Asia and their congeners across the world.

4.3. Mustela or Vison?

The American mink, together with *M. frenata*, *M. felipei*, and *M. africana*, composes a distinct New World lineage, separated first from the two weasels in Southeast Asia (*M. strigidorsa* and *M. nudipes*), and then from a larger divergent lineage of *Mustela* spanning Eurasia. This phylogenetic pattern, together with inferred genetic distances and divergence time results, provides strong evidence for generic distinction of these taxa, and a few plausible scenarios for restructuring in *Mustela* are evident, though we call for additional studies before adopting any one of them.

Coupling phylogenetic and biogeographic criteria to revise taxonomy is not unprecedented. For example, phylogenetic connections uncovered between mammals as disparate as elephants and golden moles in the superordinal clade of Afrotheria reveal an evolutionary lineage unique to the African continent, and even within the family Mustelidae, otters are separated into Old World *Lutra* and New World *Lontra* on the basis of genetic and biogeographic differences (Koepfli and Wayne, 1998, 2003; Koepfli et al., 2008). Following in a similar vein, the two primary *Mustela* clades may well represent distinct genera, with weasels and ferrets in the Eurasian clade belonging to *Mustela*, and those in the Southeast Asian-American clade being either (1) all assimilated into one distinctly new genus, or (2) species in each discrete sub-clade would take on new generic designations. With regards to the latter option, as our topology of the Southeast Asian-American grouping was not significantly different from one that associated the former weasels with the larger Eurasian clade (Koepfli et al., 2008), additional work is needed to confirm the sister relationships of the two weasels from southeast Asia. Therefore, new proposed designations for these two species will not be addressed here.

Which generic synonym would then apply to the American clade? To our knowledge, only three synonyms for *Mustela* apply to the New World species: *Vison* Gray, 1843; *Neogale* Gray, 1865; and *Grammogale* Cabrera, 1940. Priority in synonymy would render the genus name of the clade including the American weasels and the American mink as *Vison* Gray, 1843. However, Baryshnikov and Abramov (1997) stated that the European mink, *M. lutreola*, represented the type species for *Vison* (Gray, 1843), and claimed it was therefore an invalid generic designation for the American mink, calling it *Neovison vison* instead. We challenge this assumption on the basis of the specimens Gray (1843) included when he named the species. When he used the genus-group name to describe *Vison lutreola*, or the mink, he applied it to five specimens from North America and one from Siberia. As the American mink is not native to Siberia and the European mink is absent from North America, all specimens, excepting the Siberian individual, represent the animal known today as the American mink. We can only assume that Gray (1843) applied the name with the understanding that New and Old World mink were the same species, and *Vison* is clearly the oldest name exclusive to the American radiation.

To thus rename the members of the American clade (*M. vison*, *M. frenata*, *M. africana*, *M. felipei*) as *Vison*, distinct from *Mustela*, has two major effects on our understanding of New World mustelid biogeography. A new genus designation would first recognize a distinct and biogeographically coherent evolutionary lineage that diverged from Eurasian/holarctic *Mustela* during the late Miocene. Second, separating the American mink and weasels from their Eurasian counterparts would help to distinguish among weasel taxa that radiated within and are endemic to the Americas versus taxa that are descended from recent immigrations to the Americas (e.g., *M. erminea*, *M. nivalis*, *M. nigripes*).

Additionally, distinction to separate genera for species like the American and European minks or the long-tailed weasel and the ermine emphasizes the pronounced effects of ecology that act on marked genetic differences to produce similar overall phenotypes. Parallel or convergent evolution is a common phenomenon in mustelids, as evidenced by the evolution of similar phenotypes among badgers or otters, for example, and highlights that overall phenotype may be a poor indicator of true relationships. Though one might argue that the long-tailed weasel and the ermine are almost indistinguishable in the hand, genetic differences between these two quintessential weasel species demonstrate, as has been seen in earlier genetic studies of Mustelidae (e.g., skunks in Dragoo and Honeycutt, 1997; otters in Koepfli and Wayne, 1998), that molecular evidence largely overwhelms morphological evidence when it comes to revealing relationships. Placing New World weasels, like *M. frenata*, in a separate genus from Old World *Mustela*, like *M. erminea*, would remove the confusion often caused by these taxa having similar phenotypes, particularly in parts of North America where they are sympatric.

Thus, at the present time, we recommend that the most parsimonious way to resolve the phylogenetic quandary found in the relationships within *Mustela* is to separate the American mink and its endemic congeners as *Vison*. This study represents one more step towards teasing apart the true relationships within the Mustelinae, and we hope to encourage further morphological and molecular work on this group where comparisons between species will also consider divergent biogeographic histories.

5. Conclusion

Our study highlights the importance of taxonomic sampling that considers historical biogeography, as well as current species distributions, when constructing phylogenies. The position of *M. vison* within the genus *Mustela* has been debated for several decades perhaps because earlier work focused primarily on Eurasian species. While it is true that *M. vison* is highly differentiated from other *Mustela*, the American mink has rarely been compared to other endemic American *Mustela* species. When a more comprehensive representation of its American congeners is included, the appearance of a biogeographically distinct radiation of weasels in the Americas suggests the New World weasels and mink are genetically disparate from Old World *Mustela*. Thus, the placement of *M. vison*, together with *M. frenata*, *M. africana*, and *M. felipei* in the genus *Vison* outside *Mustela* is strongly supported. Though the American mink and long-tailed weasel have body plans akin to the European mink and ermine, respectively, this is likely the product of convergent evolution towards analogous lifestyles, and not due to close genetic relatedness (Youngman, 1982; Davison et al., 1999; Koepfli et al., 2008).

Taxonomic sampling of rare specimens, such as *M. africana* and *M. felipei*, proved critical to revealing genetic topology in the American *Mustela* and suggest a more ancient history than generally assumed in South America. Despite the intrinsic problems in calibration associated with estimating dates of divergence among species, this study does agree with estimated origination events of many *Mustela* species (Hosoda et al., 2000; Sato et al., 2003; Marmi et al., 2004), and adds supporting evidence to the proposed lengthy evolutionary history of mustelids in South America (Hall, 1951; Izor and Peterson, 1985). Divergence dates, particularly in the American weasel species, also reiterate the discrepancies often found between molecular histories and known fossil histories (Graur and Li, 2000), and the need to incorporate several lines of evidence to build a complete biogeographical species history.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.05.036.

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